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DENSITY-DEPENDENT GROWTH INHIBITION IN LOBSTERS, *HOMARUS* (DECAPODA, NEPHROPIDAE)[1]

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Delay of molt of juvenile lobsters (*Homarus americanus*) by earlier-molting neighbors has been demonstrated by Cobb and Tamm (Cobb, 1968, 1970; Cobb and Tamm, 1974, 1975a–c). Studies in our laboratory have indicated density-dependent inhibition, including a "nearest-neighbor" effect, upon lobster growth (Hedgecock *et al.*, 1976; Hedgecock and Nelson, 1978; Hand *et al.*, 1977). In contrast to our results, Cobb and Tamm (1975a) concluded that physical contact was necessary to produce the molt delay they observed; visual and/or chemical contact were insufficient. Such effects are of potentially great importance to crustacean aquaculture and under certain conditions may play a role in regulating growth in nature. The present paper describes an experiment with juvenile lobsters that allowed discrimination among several categories of chemically mediated growth inhibition and demonstrated a powerful short-range effect.

Materials and Methods

System design

Two semi-recirculating seawater systems, I and II, were used to culture individually-held juveniles. The systems are similar to that illustrated in Figure 2 of Hand *et al.* (1977) but include several modifications important for the study of chemical effects of animal density (Fig. 1): a) Makeup water is first treated to remove supersaturated gases. b) Returning water overflows via an adjustable standpipe at a rate equal to the makeup rate. The remainder mixes with the makeup water in sump compartment S′ where gas pressures and temperature may be regulated. Separation of sump function into two compartments offers accurate control of the makeup fraction. c) Flow through the culture tray is channeled in eight columns, each isolated by solid partitions, and each consisting of 15 compartments separated by perforated partitions. Three trays are connected in series but with intervening mixing troughs and siphon manifolds to prevent differences between adjacent columns from accumulating from one tray to the next. d) Compartment volume may be adjusted via a dam following the last mixing trough. The table design allows experimental study of changes in concentration of water-borne density-dependent factors without varying the space allotted to each animal. The gradient system of Cobb and Tamm (1974) did not allow analysis by a compartmental model (see compartmental analyses below), but was otherwise similar.

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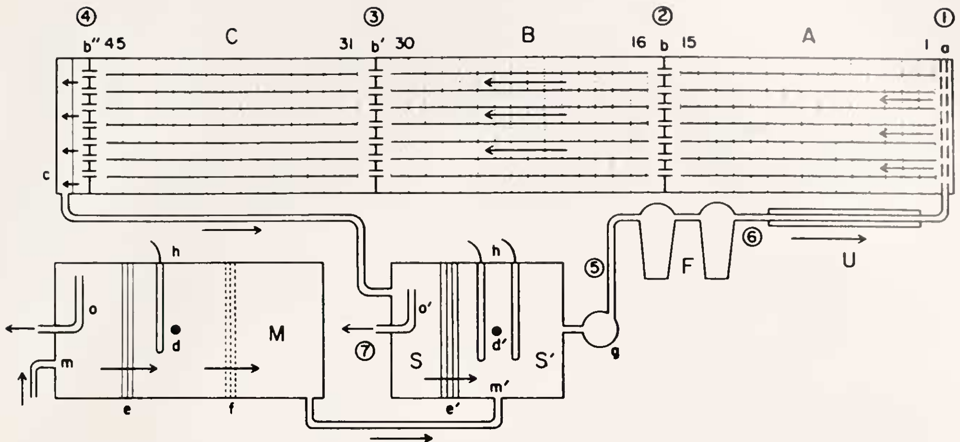

FIGURE 1. Semi-recirculating seawater system. A, B, C, culture trays; F, 25 and 5 μ canister filters; M, makeup water treatment box; S, S′, compartments of sump; U, 50 W UV water treatment unit. Details are: a, 4 l mixing trough with perforated distributor; b, b′, b″, pairs of 4 l mixing troughs joined by siphon manifolds; c, adjustable dam and return line; d, d′, airstones; e, e′, baffles; f, degassing screens; g, pump; h, heaters; m, untreated, and m′, treated makeup water input; o, o′, overflow standpipes. Circled numbers are sampling points for water quality analyses; uncircled numbers indicate rows.

Experimental subjects and protocol

Two age classes of juveniles (herein called older and younger) were used. "Older" individuals were hatched from three wild-caught *Homarus americanus* females, reared in larval systems (Hand *et al.*, 1977) until metamorphosis, and then maintained in individual compartments at ambient temperatures (*ca.* 14°C) until the beginning of the experiment, at which time virtually all were nearing the 5th-to-6th-stage molt. On Julian Calendar Day 288/78, equal numbers from each of the three females (288 in all) were randomly assigned to the spaces in rows 5–10 of each tray (numbering from the influent end). "Younger" juveniles, from a single laboratory mating of a male *H. americanus* to a female *H. gammarus*, were hatched on Day 270/78 ± 3 days and reared at 19°C until Day 288/78, at which time all were in the 4th (1st post-larval) stage. (Pure *H. americanus* individuals of appropriate age and numbers were unavailable. We are confident, however, that using hybrids did not materially affect the results reported.) A total of 432 "younger" individuals were then randomly assigned to the remaining nine rows in each tray. Backup animals for each family were maintained in another semi-recirculating seawater table at 21°C.

Within the first 7 days, 37 lobsterlings died in System I, and 55 in System II. Deaths thereafter were negligible except in rows B 10–12 of System II, where warping of the plastic tray bottom allowed cannibalization of a few molted animals. All dead lobsters were replaced and data from the replacements were used in the analysis.

Throughout most of the experiment animals were maintained on a 7 hr light: 17 hr dark photoperiod, with an additional $\frac{1}{2}$ hr of light approximately 6 hr into the dark period for a nightly check and molt collection. Lighting was from four standard 40 W clear white fluorescent tubes fixed 1.5 m above the culture trays and covered with two layers of green cellophane to reduce algal growth. Molted

carapaces were collected twice daily and their lengths recorded when possible, before they were returned to the proper compartments. Animals were fed once daily *ad libitum* with adult brine shrimp and compartments were siphoned daily before feeding. Mixing troughs and sump compartments were siphoned as needed. Cartridge filters were replaced weekly.

The experiment was terminated after 93 days, at which time weights and carapace lengths of all animals were recorded. The hybrid animals at this time were approximately 111 days old and the specimens of *H. americanus* approximately 1 month older. Only carapace length (measured from the rear edge of the orbit) and corresponding results will be reported in this paper since weight results are highly correlated with length results (Hedgecock *et al.*, 1976; Hedgecock and Nelson, 1978).

Chemical and physical analyses

Temperature, recorded thrice daily, was maintained at 21° and 20.5°C, respectively, at points 1 and 4 (see Fig. 1) with little variation between systems or from day to day. Dissolved oxygen, pH, and ammonia were measured daily at points 1 and 4, and weekly at points 2, 3, 5, and 6. Nitrate, nitrite, and organic nitrogen were analyzed weekly at sample locations 1–6. Methods of analysis for nitrogen compounds are given in Daggett and Aronstein (1979). Non-filterable residue > 0.45 μm was measured weekly at points 1, 4, and 5. Bacterial counts were made according to procedures in Standard Methods (1976) on samples taken weekly from points 1 and 4.

The systems were designed to create gradients of long-lived metabolite concentration in each system, from the influent mixing trough (sampling point 1, Fig. 1) to the effluent mixing trough (sampling point 4). By using different make-up rates but the same table flow rate (2 l/min) in the two systems, the fraction of new, non-recirculated sea water entering the table was controlled so that the gradient of long-lived metabolite concentration in System II, while no steeper, began at a threefold higher concentration. For this, the fraction of the water entering the table that was new make-up water was maintained at 50% in System I and 25% in System II. These fractions were used so that the concentrations of long-lived metabolites such as ammonia at points 1 and 4 in System I and points 1 and 4 in System II would be maintained in the approximate proportions 1:2:3:4, respectively, assuming equal *rates* of metabolite buildup in the two systems but irrespective of their actual magnitude (see compartmental analyses below). Terminal gradients from point 1 to point 4 in both systems were 50–75 μg nitrogen/l, occasionally higher. Concentrations of ammonia in System II were always considerably higher than in System I, as anticipated; the animals in the final rows of System II were occasionally exposed to concentrations greater than 500 μg nitrogen/l ($NH_3 + NH_4^+$). These levels are nevertheless very much lower than the "safe" levels suggested by Delistrary *et al.* (1977) for 4th-stage lobsters. Sampling at points 5 and 6 showed a small (< 10 μg nitrogen/l), inconsistently positive or negative effect on ammonia concentration by cartridge filtration, and a slight positive effect (< 2 μg/l) of UV treatment.

Oxygen concentration, restored to ambient values by aeration in the sump, was used as a model of the behavior of concentration of long-lived metabolites removed by filtration or aeration (see compartmental analyses below). Oxygen concentra-

tion gradients (ΔO_2) from points 1 to 4 were similar in the two systems. Only once, following pump failure, was a ΔO_2 steeper than -2 mg O_2/l observed. Terminal ΔO_2's averaged about -1 mg O_2/l. Weekly samples at points 1, 5, and 6 were near saturation and the gradient across points 1–4 was linear. A small negative gradient in pH was maintained in both systems (minimum -0.2 units, from 8.1 to 7.9 at points 1 and 4, respectively; generally much closer to zero). NO_2, NO_3, organic nitrogen, and non-filterable residue concentrations displayed no consistent gradient in either system, and very small differences from ambient values.

Bacterial counts' gradients were similar in the two systems, with values of <10/ml at point 1 and highly variable values at point 4. The means of the natural logarithms of the counts at point 4 on Tables I and II, respectively, were 7.886 ± 0.416 (s.e.) and 7.979 ± 0.518. There was no evident correlation of bacterial counts with other water quality variables, except for a general increase with time.

Following the experiment, logs of the absorbances of water samples taken 1, 2, 4, 8, and 16 min after methylene blue injection into 10 individual compartments were separately regressed on time and the slopes averaged to obtain an estimate of the first-order compartment-turnover rate, $k = 0.631 \pm 0.033$ per min. As the average compartment contained 0.25 l and the compartment flow rate was 0.25 l per min, the expected k is 1.0 per min, and mixing was thus not complete. The typical flow pattern within a compartment contained one or two gyres with vertical axes. Nevertheless, we will use a linear model with $k = 0.631$ per min in the ensuing analyses (see compartmental analyses below). In this dye test, virtually no color was observed to flow into neighboring upstream compartments, although this type of flow could have occurred during the experiment proper.

Experimental design

The block of older animals in the middle of each tray necessitates treating the design as three separate experiments (2 systems × 3 trays × k rows, with k equal to 4, 6, and 5, respectively, for the first 4 rows of younger animals, the next 6 rows of older individuals, and the last 5 rows of younger animals). While physically the experimental design is fully nested, the experimental variable is *position* (along the gradient from point 1 in System I to point 4 in System II); and we are interested in separately examining the interactions among system, tray, and row. Therefore, we treat the experiment as three three-factor, fixed-effect, factorial designs with eight replicates (Table I).

Compartmental analyses

A simple generalized catenary compartmental model (Atkins, 1969) for the concentration $a_j(t)$ of a particular metabolite in the j-th compartment at time t will be of use in discussing both the performance of the system and the growth difference results:

$$\frac{d(a_j)}{dt} = \frac{r_j}{v_j} + k_{ji}\frac{v_i}{v_j}a_i - k_{kj}a_j - k_{0j}a_j, \quad \begin{array}{l} i = j - 1 \\ j = 1, 2, 3 \ldots \\ k = j + 1 \end{array} \qquad (1)$$

where r_j is the rate of production (consumption, removal) of substance in units of mass per unit time, v_j is the volume in liters of compartment j, k_{ji} is the turnover rate (rate constant) from the preceding compartment i, and k_{0j} represents the

rate constant of loss (or gain, $+ k_{jo}a_o$) of the substance from the compartment to (or from) outside the system; all k's are in units of reciprocal time. For our purposes all k's will be assumed to be constants, although k_{oj} may be a function of the difference between a_j and a_o, the concentration outside the system.

In the case of oxygen utilization the r_j's are negative and if we assume no addition to the compartment at the air-water interface k_{jo} is zero and the right-hand term drops out. If we assume the v's and k's to be equal and that the water suffers no change in the mixing troughs, equation (1) has the steady-state solution

$$a_j = a_0 \quad \sum_{l=1}^{j} \frac{r_l}{vk} \tag{2}$$

with a_0 the ambient concentration of oxygen, $v = 0.25$ l, and k experimentally determined as 0.631 per minute. The average weight of a lobster near the end of the experiment was somewhat less than 2 g. Assuming a 2 g weight, the oxygen concentration gradient ΔO_2 from sampling point 1 to sampling point 4 (*i.e.*, a_{45} minus a_0) becomes -1.5 mg O_2/l, using the O_2 consumption value of 5.25 μg per lobster per min obtained from the regression of Logan and Epifanio (1978). Our experimental values are very close to this. The gradients in O_2 concentration were approximately linear; given the flow rates and surface–volume relationships involved, it seems safe to assume that the rate constant k_{oj} or k_{jo} for loss or gain of "volatiles" at the air-water interface were not large compared with the k_{ji}'s.

For ammonia ($NH_3 + NH_4^+$) concentration, assuming that there is loss only at the overflow o′ and that makeup water concentration is negliglible, the steady state equation (2) becomes

$$a_j = (R - 1) \sum_{l=1}^{45} \frac{r_l}{vk} + \sum_{l=1}^{j} \frac{r_l}{vk}. \tag{3}$$

R, the recirculation multiplier, is the ratio of the flow rate through the table to the makeup flow rate into the sump, *i.e.*, the reciprocal of the makeup fraction. (This R is not to be confused with the R in a similar model of Liao and Mayo, 1972, which they define as "% of water reused." See also Delistrary *et al.*, 1977.) Using an ($NH_3 + NH_4^+$) production value of 0.4 mg per lobster per day for a 2-g lobster (quoted by Moffett and Fisher, 1978, from unpublished work) we obtain a gradient from point 1 to point 4 of 79 μg/l, again consistent with our observed values. With the makeup fractions used, of course, equation (3) predicts the 1:2:3:4 ratio for a_0 and a_{45} in the two systems.

Considering only the downstream effect of a short-lived inhibitor, the appropriate compartmental model is equation (1) with r_j's greater for the older than for the younger animals (because of their larger size). We are interested only in the difference between the older and younger animals' rates of production, and therefore for simplicity we will consider the hypothetical growth-inhibiting substance as originating exclusively with the older ones and only in the last row of their block. Then the steady-state solution for the compartments downstream from the block is

$$a_j = \frac{k_2^{j-1} r_1}{v(k_1 + k_2)^j}, \quad j = 1, 2, 3 \ldots \tag{4}$$

where k_1 is the rate of loss or decay of the substance, k_2 is the turnover rate to the next compartment, r_1 is the excess production rate by the older animals and v is the compartment volume.

For $k_1 = k_2 = 0.631/\text{min}$ and $r_1/v = 1$ g per l per min we have plotted the expected steady-state concentrations (a_j) in the rows downstream from the older animal row ("row 1") in Figure 4B.

The downstream effect of pulsed production of an inhibitory substance, with the pulses far apart in time, may be simulated by equation (1) with the first and last terms eliminated, and with a unit impulse function as initial condition. There are repeated roots and the solutions are of the form

$$a_j(t) = A_j t^{j-1} e^{-kt} \tag{5}$$

With compartment sizes and turnover rates assumed to be equal in the different compartments, the time integrals of concentration in the different compartments will be the same. One may thus equate the time integrals of the solutions (5) for each j and solve for the A_j's in terms of the initial concentration pulse A_0. Equation (5) then becomes

$$a_j(t) = \frac{k^{j-1}}{(j-1)!} A_0 t^{j-1} e^{-kt} \tag{6}$$

which for $A_0 = 1$ g/l and $k = 0.631/\text{min}$ is plotted in Figure 4A.

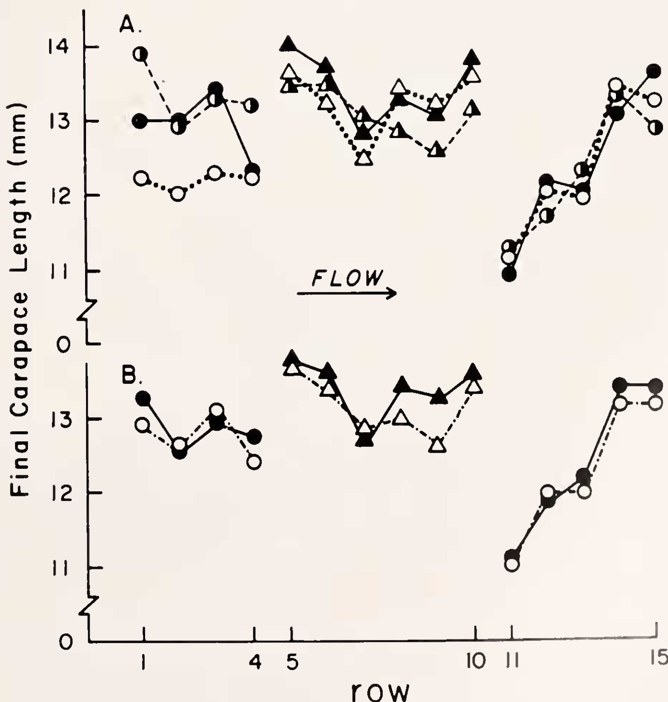

FIGURE 2. Average final carapace lengths of animals (ordinates) plotted against position in a tray (abscissa). Rows 5–10 contain the older *Homarus americanus* lobsters. In A, data from corresponding rows of the two systems are pooled. Open circles, trays A; half-filled circles, trays B; filled circles, trays C. In B, data from corresponding rows in trays A, B, and C are pooled. Open circles, System I; closed circles, System II.

Results

Final carapace length difference

Table I gives results of ANOVAs for the three "experiments." Factor 1 (System) never reaches a significant F-ratio. Figure 2B displays graphically the mean carapace–length values for each row in each system, lumping over trays; it may be seen that there is indeed no difference between Systems I and II in this respect.

Factor 2 (tray position) reaches significance ($P < 0.001$) only in the case of the first four rows of younger animals on each tray (Table IA); Figure 2A demonstrates that this is a result of a difference between the first three rows of tray A and those on trays B and C. Figure 2A illustrates also the similarity between trays for the older individuals and for the last five rows of younger animals.

The F-ratio for Factor 3 (row within tray) is highly significant for the last 5 rows of younger animals (Table IC), and significant at the $P < 0.02$ level for the rows of older individuals (Table IB). Figures 2A and B illustrate a consistent depression of final carapace length of greater than 15% in younger animals immediately downstream from the older individuals (rows 11–12), relative to the carapace lengths of those younger animals farther downsteam in rows 14–15 and also relative to those in rows 1–3 of the following tray. The corresponding depression in weight is about 40%. The second significant "row" effect is a result of a depression in older individuals' carapace lengths in the seventh, eighth, and ninth rows, relative to the fifth and tenth rows, in both systems (Fig. 2B) and at all three tray positions (Fig. 2A). Finally, when the first four rows of the last two trays only were subjected to ANOVA, the row variable just missed significance ($P < 0.064$). There is a suggestion in Figure 2A of depressed growth in younger animals of row 4 of tray C (just upstream from the block of older individuals).

We wished to determine the average rate at which the block of older animals' negative influence upon growth of the younger ones died away with distance. Accordingly, for each of the six trays, we regressed the natural logarithm of the difference between a reference value and the average carapace length in a row ($n = 8$) against its distance downstream from the older-animal block to obtain exponents for the corresponding geometric series model:

$$d_i = d_0 e^{\ li}, \quad i = 1, 2 \ldots \tag{7}$$

where d_i is the difference in mm between the carapace length for row i and the reference value, and l (in units of rows) is the reciprocal of the space constant of the series. We used a reference value of 0.1 mm larger than the largest row average in rows 11–15. The corresponding average value obtained for $\bar{l}$ was 0.690 ± 0.048. This value produces a "spatial half-life" for the downstream effect very close to one row.

Molt interval and increment differences

The 15% decrement in growth rate appears to be partitioned approximately equally between an increase in intermolt interval and a smaller molt increment. Carapaces, especially of the smaller molts, were often partially eaten before they were retrieved and measured, and many molts were missed, so that molt time information also was often lacking or uncertain. Hence, we subtracted the row

TABLE I

Analysis of variance, final carapace lengths.

Source	Degrees of freedom	Sum of squares	Mean square	F-ratio	P[1]
A. First four rows of younger animals (rows 1–4)					
Total	191	326.01			
Replicates	7	7.24	1.03	0.69	
System (1)	1	0.57	0.57	0.38	
Tray (2)	2	41.04	20.52	13.73	0.000
Interaction 12	2	3.35	1.92	1.29	>0.25
Row (3)	3	9.26	3.09	2.06	>0.10
Interaction 13	3	2.82	0.94	0.63	
Interaction 23	6	10.06	1.67	1.12	>0.35
Interaction 123	6	10.55	1.76	1.18	>0.30
Error	161	240.64	1.49		
B. Six older animal rows (rows 5–10)					
Total	287	599.73			
Replicates	7	16.29	2.33	1.14	>0.30
System (1)	1	4.72	4.73	2.32	>0.10
Tray (2)	2	7.68	3.84	1.89	>0.15
Interaction 12	2	4.15	2.08	1.02	>0.35
Row (3)	5	29.71	5.94	2.92	0.02 > P > 0.01
Interaction 13	5	4.20	0.84	0.41	
Interaction 23	10	10.40	1.04	0.51	
Interaction 123	10	23.80	2.37	1.17	>0.30
Error	245	498.76	2.04		
C. Last five rows of younger animals (rows 11–15)					
Total	239	652.32			
Replicates	7	9.47	1.35	0.63	
System (1)	1	1.86	1.86	0.88	
Tray (2)	2	0.70	0.35	0.16	
Interaction 12	2	2.35	1.17	0.55	
Row (3)	4	177.75	44.44	20.89	0.000
Interaction 13	4	1.26	0.32	0.15	
Interaction 23	8	8.95	1.12	0.52	
Interaction 123	8	16.36	2.05	0.96	
Error	203	433.61	2.14		

[1] Value only given for F > 1.0.

average for a particular molt number from that of the preceding one to estimate the interval or increment. For this reason standard errors are given only for molt time and carapace length in Table II, which summarizes the differences between the first and fifth rows of younger animals (*i.e.*, rows 11 and 15) following the older animal block in each tray.

The "nearest-neighbor effect" is felt immediately. Animals immediately downstream of the older individuals molt from fourth to fifth stage a day and a half later than those five rows downstream, and when their next molts are measured are found to be already 5 percent smaller. The quotient (b/a) combining the

TABLE II

Growth differences between first and fifth rows following the block of older animals, as reflected in intermolt interval and molt increment. Columns (a) and (b) express age and carapace length, respectively, of row 11 animals as percent of those in row 15. The last column estimates growth of row 11 animals as percent of those in row 15. Based upon final carapace length measurements this was 83.7%.

Molt to stage	Row	N	Age at molt (days)[1] $\bar{x} \pm$ s.e.	(a)	Intermolt interval (days) ($\bar{x}_i - \bar{x}_{i-1}$)	N	Carapace length after molt (mm) $\bar{y} \pm$ s.e.	(b)	Molt increment (mm) ($\bar{y}_i - \bar{y}_{i-1}$)	Estimated growth ($\frac{b}{a} \times 100$)
5	11	40	28.88 ± 0.39	105.7		22	5.355 ± 0.076	95.5	0.567	90.4
	15	34	27.33 ± 0.31			20	5.610 ± 0.089		0.810	
6	11	31	42.69 ± 0.44	107.81	13.81	21	6.256 ± 0.105	91.0	0.901	84.4
	15	41	39.60 ± 0.35		12.27	20	6.875 ± 0.124		1.265	
7	11	32	55.41 ± 0.60	107.25	12.70	20	7.590 ± 0.193	92.6	1.334	86.3
	15	40	51.66 ± 0.40		12.04	26	8.192 ± 0.122		1.317	
8	11	38	71.37 ± 0.50	107.63	15.96	23	9.083 ± 0.202	91.0	1.493	84.5
	15	42	66.31 ± 0.62		14.65	20	9.985 ± 0.238		1.793	
9	11	26	89.00 ± 1.01	107.0	17.63	12[2]	10.025 ± 0.336	85.5	0.942	79.9
	15	38	83.21 ± 0.77		16.90	27	11.719 ± 0.234		1.734	

[1] Taking Day 270/78 as age 0.
[2] Molt to stage 10 incomplete for this row by end of experiment.

effects of molt increment decrease (b) and molt interval increase (a) shows by the second molt in the system the full 15% decrease in growth seen in the final carapace-length figures. Upon exposure to 5th- to 6th-stage juveniles, the 4th-stage lobsterling apparently switches very soon to a slower rate of growth, reflected in both intermolt interval and molt increment.

DISCUSSION

Results of this study permit further elucidation of the density-dependent growth inhibition observed in earlier experiments with juvenile lobsters. The experimental design allowed separation of the effects upon growth of a) long-lived metabolites not removed by filtration, air-stripping, etc., which would thus build up along the table, and be much higher in concentration in System II with its lower makeup rate (see compartmental analyses above); b) long-lived metabolites removed by air-stripping or other means, which would build up along a table, but similarly in the two systems; and c) metabolites whose effects in the system were short-lived. The gradients in ammonia concentration may serve as a paradigm of a). Were such a metabolite effective in inhibiting growth in the concentrations attained, we would expect system differences, tray differences, and row differences to be manifest in the ANOVAs of Table I. Similarly, were metabolites which were removed in the sump, filters, or UV units effective in inhibiting growth in this experiment, we would expect only tray differences and row differences to appear in the ANOVAs. In fact, there were no differences at all between the systems, a result similar to that obtained by Cobb and Tamm (1974). The only tray differences were between the first three rows of trays A and the first three rows in trays B and C (Fig. 2A). (Surprisingly, growth in these first three rows in the gradient was *slower*. We have no ready explanations for this, although several possibilities exist. First, it is possible but not likely that supersaturation of gases in the seawater influent was not entirely removed by heating, air-stripping, and nucleation in the makeup

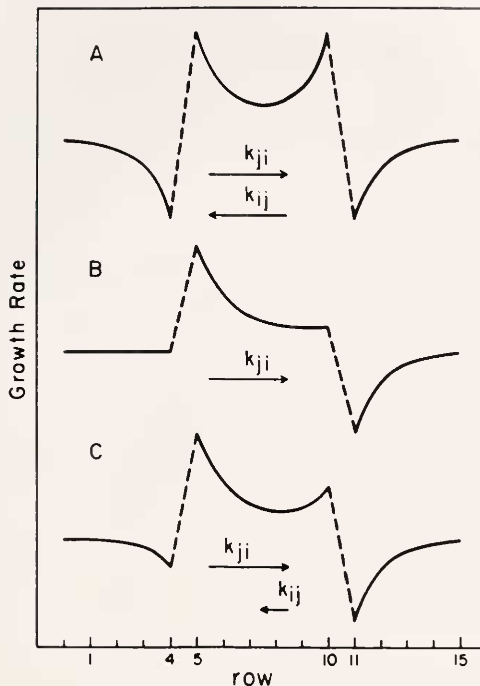

FIGURE 3. Compartment models corresponding to Figure 2. Growth rate (ordinate, arbitrary units) is plotted against compartment number in a catenary array (abscissa). Production of inhibitory principle is higher in compartments 5–10. In A, the turnover rate constants k_{ji} and k_{ij} (see compartmental analyses) are symmetrical; in B, the k_{ij}'s are negligible; in C the k_{ij}'s are small but not negligible.

sump (Fig. 1, M). Secondly, the UV treatment could have resulted, for example, in the production of short-lived ionized molecules with an adverse effect upon growth. Third, it is possible that "natural" sea water, either because of the presence of a substance removed by lobsters or through the absence of a "conditioning" substance produced by lobsters, is not as conducive to growth of lobsters as is lobster-conditioned water (see Cobb and Tamm, 1975a). But if the effect in our systems were attributable to the makeup water, it should be more pronounced in System I with the higher makeup fraction, which a separate two-factor factorial ANOVA on just the first four rows of trays A failed to show. Therefore, if a "conditioning" substance is responsible, it must be removed from the recirculation water before the latter re-enters tray A.)

Density-dependent growth inhibition appeared instead to be a short-lived effect of animals in the immediate neighborhood, *i.e.*, an effect which dies away with distance, and was obviously asymmetrical, *i.e.*, more pronounced downstream. Figure 3A indicates schematically what we would expect from a compartmental model (see compartmental analyses above) of a 15-compartment column with symmetrical upstream and downstream turnover constants k_{ij} and k_{ji} for the transmission of a short-lived inhibitory influence. Figure 3B shows the pattern anticipated were the upstream flow completely negligible, and Figure 3C the pattern with dominant downstream flow but non-negligible upstream influence. In the case of Figure 3A growth would be stunted symmetrically in the younger individuals on either side

of the block of older animals, and also in the middle of that block. The reason for the latter effect is that the older animals at the edges of the block have only half the number of large neighbors as do the ones in the middle of the block and hence experience less inhibition. With model 3B only the older animals immediately downstream from the younger animals would be "released" from inhibition, and only the younger animals immediately downstream from the larger ones inhibited. If there were some slight upstream range of the inhibitory influence, the pattern of Figure 3C would result.

Comparison of Figure 3 to Figure 2 suggests the model of Figure 3C, and appears to eliminate certain explanations. First, the possibility of a direct behavioral cause, for example aggressive interactions, appears to be eliminated by the transmission of the effect to non-adjacent compartments. It is hard to imagine an effect based upon visual or bodily contact which could produce this result. Moreover, radiative influences (*e.g.*, sound) cannot account for the upstream-downstream asymmetry. We appear to be left with some form of chemical influence, carried differentially downstream, *but not accumulating*. Although in the dye tests no significant backflow of dye was observed, the possibility of small but appreciable upstream influence cannot be excluded.

There appear to be only two tenable hypotheses: first, the continuous production of a chemical substance or complex whose effect is short-lived, either because of loss to the outside or because of decay or inactivation within the system; and second, the pulsed production of a more or less long-lived inhibitory substance. Either process would be capable of producing an effect upon growth which died away with distance from the source. We will examine the two hypotheses in turn.

For a short-lived inhibitor with decay rate equal to the compartment turnover rate $k = 0.631$ per min, the steady-state concentrations in compartments downstream from the older-animal source may be calculated to be as shown in Figure 4B (see compartmental analyses above). For comparison we recall that the regressions for the downstream decay of the nearest-neighbor effect gave "spatial half-lives" of approximately one row for the downstream effect, as with these parameters. Thus we may say that if the growth inhibitory effect is due to a molecule or complex which decays or is otherwise lost from the system, the decay constant is on the order of 0.6/min or that the substance has a half-life of approximately 1 min. It appears from the oxygen results (see compartmental analyses above) that it is extremely unlikely that a substance could be lost through the air–water interface at such a rate, and surface absorption or bacterial inactivation at that rate seems similarily unlikely. If the molecule or complex is indeed lost from the system at this rate, it appears that it is unstable or easily inactivated by other factors in seawater.

If the inhibitor is produced in a pulse, as for example with intermittent urinary production (Cornell, 1979), the concentration pulse will "spread out" and become lower as it travels downstream, as in Figure 4A (using the compartment turnover rate $k = 0.631$/min; see compartmental analyses). With the pulsed-production model the question arises: To which aspect of the concentration is the downstream animal sensitive? If it integrates the concentration over time, then each downstream animal will experience the same quantitative effect upon growth. But if it is sensitive instead only to the maximum concentration experienced, or to the rate of increase in concentration in its compartment following the unit impulse upstream (in effect differentiating the curve of rising concentration), downstream effects upon growth rate similar to those observed could be obtained. Whatever

parameter of the pulse is presumed effective, if its duration is much greater than a minute, or its onset is less abrupt than the unit impulse, downstream smoothing will be more pronounced and the successive compartments less differentiated from one another. Pulsed release of urine containing an inhibitor is a likely candidate. However, the hypothesis of a continuously produced short-lived molecular species or complex with a half-life on the order of a minute appears at this time to be equally tenable.

Without the blocks of older animals in the experiment, this rapidly-decaying effect upon growth would not have been demonstrated. However, that growth in the middle of these blocks is inhibited relative to the lead row of the block (Fig. 2) indicates that we are not just dealing with an effect of older upon younger animals, or of *H. americanus* upon hybrids. Even with cohorts of similar sized individuals such growth inhibition may be presumed to exist, and removal of it should result in economically significant gains in growth rate. But the best way to go about this will depend on experimental answers to the questions a) Is the inhibitory substance pulsed in its release, rapidly decaying, or both? and b) Is the substance self-inhibitory?

The fast-decaying growth inhibitory effect we have observed apparently differs in several respects from the delay-of-molt phenomenon described by Cobb and

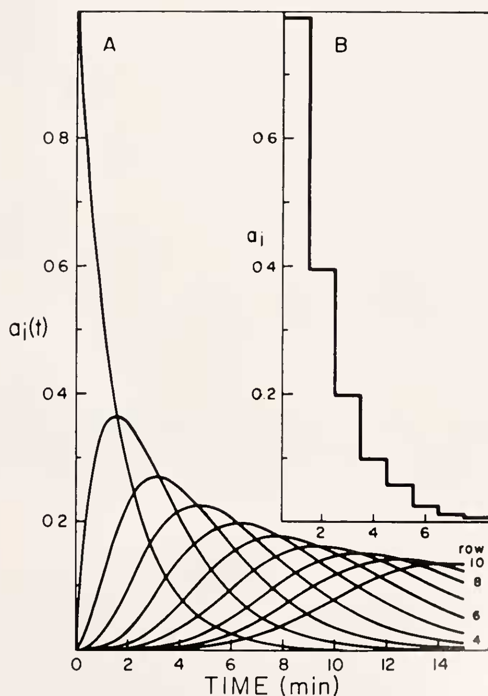

FIGURE 4. A. The course of concentration (ordinate) with time following a unit impulse of production (abscissa), in the successive compartments (rows) downstream from the production compartment (separate curves; the exponential curve represents the clearance of the production compartment). $A_0 = 1$ g per 1; $k = 0.631$ per min. See compartmental analyses.

B. Steady-state concentrations (ordinate) in successive rows (abscissa) downstream from a point of continuous production of an inhibitor decaying with a rate k_1 equal to the turnover rate to the next compartment k_2, both equal to 0.631/min. The rate of production has been set equal to 1 g per 1 min. See compartmental analyses.

Tamm (Cobb, 1968, 1970; Cobb and Tamm, 1974, 1975a–c). In their experiments the dominant individual of a pair held together clearly delays the molt of the subordinate individual, but no mention is made of an effect upon the growth increment of the subordinate. Furthermore, the molt-delay phenomenon is said to disappear in the absence of physical contact (Cobb and Tamm, 1975a). It seems instead to be an expression of dominance, possibly brought about in part by the greater activity of the subordinate animal (Cobb and Tamm, 1975c; the effect on growth which we have found could of course also be brought about by increased activity of the downstream animals). However, the molt-delay phenomenon is not clear in groups of three or more lobsters (Cobb and Tamm, 1975b). In further contrast to our results is their suggestion that chemical communication in the absence of visual or physical contact may facilitate molting rate and synchrony, although the evidence is apparently not convincing (Cobb and Tamm, 1975a). Our results point instead to a desynchronizing influence of the fast-decaying inhibitory effect. However, the possibility of chemical facilitation of growth (need for "lobster-conditioned" water) was discussed above as an explanation of the reduced growth rates in the first three rows of tray A (Fig. 2A).

The fast-decaying inhibitory effect together with dominance effects may account in full for the enhancement of growth rate variance in various crustaceans held communally (Cobb and Tamm, 1975b; Malecha, 1977; Aiken, 1977). In mass culture the dominants' behavior tends to isolate them from the more subordinate animals, which in turn often tend to crowd together in the corners, etc. (pers. obs.). Under such conditions a fast-decaying or pulsed inhibitor could exert maximum effects upon the subordinates. Whether such massing together occurs in nature is unknown. But it may under certain circumstances, *e.g.*, spatially concentrated food or shelter. Some of the so-called "space limitation" effects in crustacean culture experiments (Rauch *et al.*, 1974; Shleser, 1974; Sastry *et al.*, 1975; Sastry and French, 1977; Van Olst and Carlberg, 1978) may also be due to a fast-decaying inhibitory effect in compartment arrays which have not been designed to differentiate between the effects of limited space *per se* and the effects of nearest-neighbor inhibition (see also Cobb and Tamm, 1975c).

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Summary

1. A density-dependent inhibitory effect upon the growth of juvenile lobsters was studied in two semi-recirculating seawater-table systems in which controlled metabolite gradients were established by varying rate of makeup flow. Each system contained eight columns of 45 compartments each. *Homarus americanus* fifth-stage juveniles were placed in the compartments in rows 5–10, 20–25, and 35–40, numbering from the incurrent end of each system, and fourth-stage *H. americanus* × *H. gammarus* F_1 hybrids were placed in all other compartments.

2. At the end of 93 days hybrids immediately downstream from the older animals were an average of 15% shorter than those five rows downstream. No effect of metabolite accumulation could be demonstrated.

3. Analysis by a compartmental model indicated that the growth-inhibitory effect was chemical and was due either to a rapidly decaying, continuously produced inhibitor with a half-life of about 1 min, or to an inhibitor pulsed in production or release.

4. Decreased growth in the middle of the blocks of older animals demonstrated that the effect was neither species- nor age-specific. The effect is substantially different from other growth-inhibitory phenomena previously described in Crustacea.

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